Comparison of Irrigation Solutions and Devices in a Contaminated Musculoskeletal Wound Survival Model

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Background: There is much to learn about the effectiveness of different methods currently used for the irrigation of open wounds. The purpose of this study was to compare various approaches in a survival animal model.

Methods: We used an established goat model involving the creation of a reproducible complex musculoskeletal wound followed by inoculation with *Pseudomonas aeruginosa* (lux) bacteria. This genetically altered luminescent bacterium provides the ability for quantitative analysis with a photon-counting camera system. For Study 1, wound irrigation was performed six hours after the injury and inoculation; the goats were assigned to four treatment groups: normal saline solution, bacitracin solution, castile soap, and benzalkonium chloride. All wounds received sharp débridement and irrigation with use of a pulsatile lavage device (19 psi). Images and photon counts were obtained prior to irrigation, after irrigation, and forty-eight hours after injury and inoculation. For Study 2, we used the same animal model and compared bulb syringe and pulsatile lavage irrigation with saline solution.

Results: In Study 1, the irrigation treatment lowered the bacterial counts in all treatment groups. The greatest reduction was seen with castile soap, which lowered the photon count to 13% of the pretreatment level. This was followed by benzalkonium chloride, bacitracin, and saline solution at 18%, 22%, and 29%, respectively. At forty-eight hours, imaging showed a rebound in bacterial counts in every group. The highest rebound was measured in the castile soap group, which rebounded to 120% of the pretreatment level. The benzalkonium chloride group experienced a rebound to 94% of the pretreatment level. These were followed by bacitracin solution (89%) and normal saline solution (68%). In Study 2, both treatment methods were effective in removing 75% of the bacteria initially. At forty-eight hours, the bacterial levels in the pulsed lavage group rebounded to 94% of the original levels (compared with 48% in the bulb syringe group). The difference in the mean photon count ratios at forty-eight hours was significant ($p = 0.048$).

Conclusions: Approaches used to remove bacteria from wounds, such as irrigants other than saline solution or high-pressure devices, may not have the best clinical outcome.

Clinical Relevance: These data suggest that use of a low-pressure device and saline solution to irrigate wounds is the best choice.

In a contaminated open fracture wound, the quantity of bacteria present has been shown to correlate with the risk of infection[1,2]. Therefore, one of the goals of the initial treatment of open fracture wounds is to decrease the bacterial load and remove as much necrotic tissue as possible. The actual amount of bacteria in a contaminated wound that would result in a clinical infection has not been determined and is dependent on many factors such as the severity of the wound and the health status of the host. Methods that reduce bacterial counts are considered advantageous in the management of contaminated wounds and open fractures.

The use of many different solutions has been proposed to increase the quantity of bacteria removed by wound irrigation. There is little evidence that the use of antiseptic solu-

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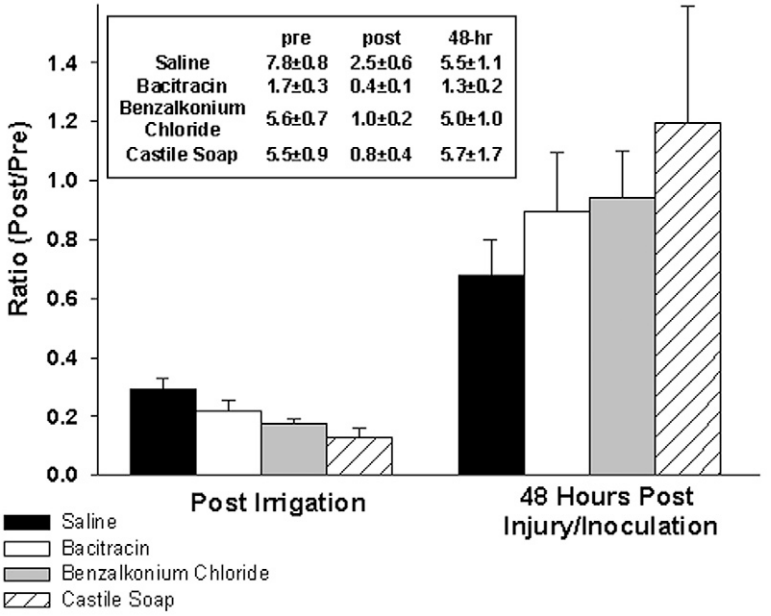

Fig. 1

Comparison of the mean ratio of the luminescent bacteria remaining in the wound immediately after irrigation and at forty-eight hours after the injury-inoculation to the pre-irrigation level. The table inset shows the mean photon counts (and the standard error of the mean) ($\times 10^5$) at the three time points.

tions offers a substantial benefit to the cleaning of traumatic wounds. While the bactericidal properties of antiseptic solutions have been noted in vitro, the clinical use of such solutions results in host tissue necrosis and is therefore not recommended[1]. Surfactants and antibiotic additives have shown efficacy in animal studies[3-6]; however, clinical trials are limited[7].

Unfortunately, there is not a clear choice for irrigation devices either. Currently, pulsed lavage units are commonly used as a means to effectively lower the bacterial counts in contaminated wounds associated with open fractures[3,7]. While evidence has shown that these devices are effective at removing bacteria[8], there are concerns about the effects on host tissues because of the higher pressures[9-13].

The goal of Study 1 was to compare three alternative irrigation solutions with the standard of care (sterile normal saline solution) in an established large-animal model. We chose to evaluate benzalkonium chloride, castile soap, and bacitracin solutions. Each of these solutions has been shown to be efficacious in previous studies[3-6]. Our hypothesis was that the wounds that were irrigated with these alternative solutions would have fewer viable bacteria immediately after irrigation and forty-eight hours after inoculation than would the wounds irrigated with saline solution. During Study 1, we noticed a profound rebound in the amount of bacteria toward the baseline values within the wound by forty-eight hours after inoculation. We hypothesized that the higher pressure of the pulsatile lavage was causing this effect. The goal of Study 2 was to compare the effectiveness of bulb syringe with pulsatile lavage irrigation. Although pulsatile lavage is capable of removing more bacteria from wounds[8], we hypothesized that the higher pressure may actually cause a worse outcome. We used the amount of bacteria within the wound at the forty-eight-hour time point as our outcome measure. This is typically when wounds are débrided a second time, if required.

Materials and Methods

We modified a large-animal model of a contaminated musculoskeletal wound that was established in previous work at our laboratory[8]. All procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited laboratory after obtaining protocol approval from the Institutional Animal Care and Use Committee. After the goat was anesthetized, the left hind limb was shaved, aseptically prepared, and draped. An 8-cm skin incision was created parallel to the crest of the tibia in the midportion of the medial aspect of the tibial fascia. A combination of blunt and sharp dissection was utilized to expose the periosteum and investing fascia of the anterior and lateral compartments. Electrocautery was used to incise the periosteum at the level of the tibial crest for 5 cm. A parallel incision was made medially through the periosteum, leaving a 6-mm intact strip of periosteum on the anterior aspect of the tibia. The periosteum was elevated with electrocautery to expose the medial aspect of the tibia to the posteromedial ridge and the attachment of the posterior compartments medially. The fascia was elevated from the anterior compartment, exposing the muscles in the compartment. A 3-mm drill-bit on a twist drill and a small osteotome were utilized to create a 1.2-cm-diameter

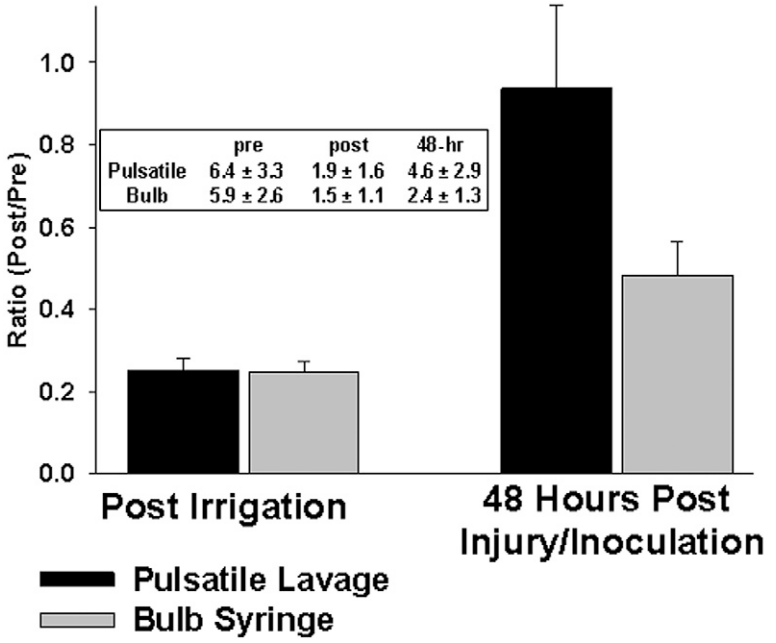

Fig. 2

Comparison of the mean ratio of the luminescent bacteria remaining in the wound immediately after irrigation and at forty-eight hours after the injury-inoculation to the pre-irrigation level. The table inset shows the mean photon counts (and the standard error of the mean) ($\times 10^5$) at the three time points.

cortical defect, avoiding breach of the cortical wall. Three Kelly clamps were applied and spaced evenly over 5 cm of the exposed anterior compartment muscle and were left in place for three minutes to induce a crush injury. Concurrently, electrocautery was used to create thermal damage to the intervening exposed muscle, the overlying fascia, and the retracted medial periosteum. This resulted in a consistent complex musculoskeletal wound involving injury to muscle, fascia, periosteum, and bone.

The wound was inoculated with 1 mL of $>10^8$ CFU/mL of *Pseudomonas aeruginosa* (lux), spread evenly over the wound surfaces with a cotton-tipped applicator. This strain of bacteria has been genetically modified to emit light[8]. The wound was left open for a five-minute period after which the wound was stapled closed. Two 5-mm Schanz pins were placed in the proximal aspect of the tibia in a percutaneous manner, and a dressing was applied.

After surgery, the goat recovered in its pen for six hours and was allowed activity ad libitum. Six hours after surgery, general anesthesia was again induced. The goat was placed supine on the operating table, and the left lower extremity was mounted to the camera with an external fixation frame and the previously placed Schanz pins. The extremity was then aseptically prepared and draped. A photon-counting camera (Charge Couple Device [CCD] Imaging System model C2400; Hamamatsu Photonics, Hamamatsu-City, Japan) was used to capture the quantitative and spatial distribution of the bacteria in the wound. A black-and-white image was obtained, and a photon count of the region was performed. Once the baseline luminescent data were collected, treatment was rendered. The model and validation with quantitative measurements have been described previously[8].

For Study 1, the thirty-two (45 to 55-kg) castrated male goats were assigned to four treatment groups: castile soap, benzalkonium chloride, bacitracin, and normal saline solution. The castile soap solution was prepared by adding approximately 80 mL (sixteen packets) of nonsterile, liquid castile soap (Triad Medical, Franklin, Wisconsin) to each 3-L bag of normal saline solution. The benzalkonium chloride solution was prepared by injecting 5.29 mL of 17% benzalkonium chloride (EMD Biosciences, San Diego, California) to each 3-L bag of normal saline solution. The bacitracin solution was prepared by injecting 100,000 U of bacitracin (Pharma-Tek, Huntington, New York) to each 3-L bag of normal saline solution. After sharp débridement of necrotic tissue, 6 L of irrigant were administered by means of a pulsatile lavage device (InterPulse Irrigation System; Stryker Instruments, Kalamazoo, Michigan) operated at its highest setting. This pulsatile lavage system used a high-flow tip attachment (model 210-14) with a maximum pressure of 19 psi and a maximum flow rate of 1025 mL/min.

After treatment, repeat images of the wounds were made. The wound edges were stapled closed, and a sterile dressing was applied. The animals recovered in their pens and were allowed activity ad libitum. Forty-eight hours after the wound inoculation procedure, the animals were killed and repeat imaging of the wound was performed.

TABLE I Commercially Available Pulsed-Lavage Systems

Name	Company	Pressure *(psi)*	Studies
InterPulse*	Stryker	6-19	Svoboda et al.[8] and Draeger and Dahners[12]
Surgilav Plus	Stryker	14-70	Bhandari et al.[9,14], Boyd and Wongworawat[13], Caprise et al.[28], and Hassinger et al.[26]
Pulsavac	Zimmer	9-22	Tabor et al.[25]

*The device used in the present study.

Raw data were collected in the form of photon counts generated by the CCD camera and image processor. For the photon counts, the AquaCosmos imaging software (Hamamatsu Photonics) provided a photon count for the entire field within view of the camera. Ratios of photon counts for each animal at each time point compared with the baseline photon-count level were calculated. All ratios were analyzed between treatment groups with use of a hierarchical mixed-model analysis of variance allowing for treatment, time, and the interactions among treatment and time as fixed effects and replicate study as a random effect. Preplanned orthogonal contrasts between the treatments at each time point were conducted. The level of significance was determined as $p < 0.05$. All values are reported as the mean and the standard error of the mean.

During Study 1, we noticed an increase in bacteria from the imaging made after débridement to the imaging made at forty-eight hours. We hypothesized that this rebound in bacteria toward pre-irrigation levels may have been caused by the pulsatile lavage device. A power analysis determined that twelve animals per group were required to detect a 50% difference between groups. Study 2 evaluated saline solution irrigation by means of pulsatile lavage and bulb syringe. We used the same procedure from Study 1 for the pulsatile lavage group and utilized the data from the eight animals from the saline solution group in an effort to reduce the number of animals. The bulb syringe group received 9 L of saline solution applied to the wound with use of a bulb syringe (Kendall, Mansfield, Massachusetts). The different quantities of saline solution used were based on previous work[8] and pilot work, which demonstrated that these amounts resulted in similar bacterial counts after irrigation.

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No outside funding was received for this study.

Results

The irrigation and débridement lowered the bacterial counts prior to treatment for all treatment groups (Fig. 1). The greatest reduction was seen with castile soap, which lowered the photon count to 13% ± 3% of the pretreatment level. This was followed closely by benzalkonium chloride (18% ± 2%). Bacitracin solution and saline solution lowered the bacterial counts to 22% ± 4% and 29% ± 4%, respectively. The reduction with castile soap was significantly greater than that with the normal saline solution ($p = 0.0069$), while the reductions with benzalkonium chloride ($p = 0.079$) and bacitracin ($p = 0.30$) were not.

At forty-eight hours, imaging showed a rebound in bacterial counts in every group. The highest rebound was measured in the castile soap group, which rebounded to 120% ± 40% of the pretreatment level. The benzalkonium chloride group experienced a rebound to 94% ± 16% of the pretreatment level. These were followed by bacitracin solution (89% ± 20%) and normal saline solution (68% ± 12%). The bacterial levels at forty-eight hours were significantly higher than the levels after irrigation for the castile soap ($p = 0.0001$), benzalkonium chloride ($p = 0.0032$), and bacitracin ($p = 0.0082$) groups.

In Study 2, sharp débridement and irrigation removed the majority of the bacteria from the wound (Fig. 2), with only 25% of the pretreatment bacterial levels seen after irrigation in both groups. At forty-eight hours, the photon counts from the bacteria were higher ($p = 0.048$) in the wounds that received pulsed lavage irrigation than in the wounds that received bulb syringe irrigation (94% ± 20% and 48% ± 8% of the pre-irrigation levels, respectively).

Discussion

In our model, none of the tested solutions performed better than normal saline solution. While castile soap removed significantly more bacteria during wound irrigation, there was a rebound at forty-eight hours in the mean bacterial count to a level higher than that before initial treatment. Benzalkonium chloride and bacitracin did not remove significantly more bacteria than normal saline solution; both also experienced a significant increase in bacterial counts at forty-eight hours. Normal saline solution, while removing the least initially, had the smallest increase in bacterial counts at forty-eight hours. While alternative solutions may provide improved bacterial removal with wound irrigation, it appears that this increase in ability may not be as beneficial as once thought. This may be explained by an increase in host tissue destruction associated with these solutions; however, our study did not provide any histological analysis of the host tissue.

There has been a substantial amount of basic-science and translational work done on alternative irrigation solutions. In vitro work by Bhandari et al. tested the viability of mice calvarial cells on exposure to various solutions: normal saline solution, ethanol (1% and 10%), povidone-iodine (1%), chlorhexidine gluconate (1% and 4%), liquid soap (1% and 10%), and bacitracin (50 U/L)[14]. All solutions resulted in a decrease in osteoblast and osteoclast viability compared with normal saline solution. Anglen et al. showed improved efficacy of bacitracin

(50,000 U/L) and castile soap (18 mL in 1 L of normal saline solution) over normal saline solution when irrigating *Staphylococcus epidermidis* (eighteen-hour incubation in broth) from stainless steel hardware[4]. This group continued to evaluate the efficacy of various solutions to remove various bacterial strains from inorganic materials and cadaver tissues and found improved efficacy with castile soap, bacitracin, and benzalkonium chloride[3,5,6]. With the use of a rat dorsal wound model (fifteen-minute inoculation)[15,16], they developed a sequential combination irrigation with castile soap, normal saline solution, and benzalkonium chloride that was superior to irrigation with each individually and normal saline solution alone[17,18]. This group summarized their body of work and recommended the use of surfactants such as benzalkonium chloride and castile soap[19].

Quality clinical trials of irrigation solutions are scarce. Anglen performed a prospective randomized trial of 400 patients with a lower-extremity open fracture receiving irrigation with either bacitracin solution or castile soap solution[7]. He found no difference in the number of infections, but he did note more wound-healing problems in the bacitracin group. The absence of a normal saline solution group does not allow an important comparison and prevents one from being able to determine the optimal irrigant.

The current work demonstrates that three alternative solutions did not perform any better than normal saline solution in a clinically relevant large-animal extremity model. We cannot determine whether these results will make a difference in a clinical scenario. One potential concern when trying to interpret these data is the use of *Pseudomonas aeruginosa* with benzalkonium chloride solution. The use of benzalkonium chloride solution on a wound contaminated with *Pseudomonas aeruginosa* demonstrated an increase in infection and wound complications in a previous animal study[20]. While this reaction was not noted in an earlier study comparing benzalkonium chloride and other irrigation solutions in a polymicrobial-inoculated fascial wound in a rat model, a clear increase in efficacy against *Staphylococcus aureus* was demonstrated[21]. Development of a luminescent model with use of *Staphylococcus aureus* is in progress, and it would allow us to compare these results with a gram-positive bacterial strain. The gram-positive bacteria do not currently luminesce at a level that allows us to use them.

Recent studies have suggested that high-pressure lavage may not be the ideal way to irrigate wounds. Our study supports this idea. A comparison of irrigation with 6 L of normal saline solution by means of pulsed lavage and 9 L of normal saline solution by means of bulb syringe with use of our contaminated wound model showed similar initial reduction in bacterial counts ($p = 0.90$). However, after forty-eight hours, a significant increase in bacteria was seen in the pulsed lavage group compared with the value after irrigation ($p = 0.0003$). This increase in bacterial growth in the period after treatment suggests that pulsed lavage irrigation may not be the best way to deliver saline solution. It should be noted that this result is different from a previous study that used the same contaminated musculoskeletal wound model to compare the effectiveness of pulsed lavage and bulb syringe irrigation[8]. The previous study demonstrated that pulsed lavage removed more bacteria from the wound; however, the animal was not recovered for the forty-eight-hour evaluation and the authors were not able to assess the effect of the higher pressure on the tissue and the bacterial growth after the treatment. Another difference is that the wounds in the previous study did not receive sharp débridement. We thought that it was imperative to débride the wound in a survival model such as this. The previous study determined the effectiveness of irrigation devices on removing bacteria from the wound. We believed that débriding the wounds in that study would have masked the difference in bacterial removal between the irrigation devices. We believed that not débriding necrotic tissue, which is the clinical procedure, in this survival model could have potentially masked the effect of the irrigation devices at a later time point. This underscores the importance of selecting the appropriate animal model to evaluate irrigation devices and clinical guidelines and the conclusions that one can draw from such studies.

The study and use of pulsed lavage systems can be confusing or misleading because several different irrigation systems that produce a wide range of pressure are available for use. To compound the problem, there is inconsistency within the literature for what qualifies as high-pressure and low-pressure irrigation[9,13,14,22]. For example, early research in this area defined high pressure as 7 to 20 psi[23] and 10 to 15 psi[24]. However, more recent reports have favored a classification of pressures in the range of 35 to 70 psi as high pressure and 1 to 15 psi as low pressure[9,13,14,22]. Bahrs et al. referred to pressures in the range of 10 to 30 psi as medium pressure[25], and Tabor et al. defined high pressure as 28 psi[26]. A recent study by Draeger and Dahners used a device that generated 6 to 19 psi and labeled it as high pressure[12]. Table I lists some of the recently studied lavage systems and their manufacturer-reported pressures. We chose the Stryker InterPulse lavage device as it is currently the most utilized pulsatile lavage unit by deployed U.S. Army medical units. We used it on its highest setting with a high-flow tip attachment in order to produce a maximum pressure of 19 psi. In light of this literature, we consider this to be an intermediate-pressure device.

Some authors have encouraged the use of pulsed lavage on the basis of animal studies. Brown et al. conducted one of the first evaluations of pulsed lavage irrigation in an animal model[27]. They used a rat dorsal wound with crushed muscle inoculated with *Escherichia coli* and treated immediately with 300 mL of normal saline solution by means of a bulb syringe, gravity flow, and pulsed lavage at 50 psi. The pulsed lavage-treated wounds had lower bacterial counts at three, seven, and ten days without the rebound seen in our model. Caprise et al. examined pulsed lavage in a rabbit open fracture model and found that, with the addition of an inoculum and foreign material into a fracture site, the group treated with pulsed lavage (Surgilav Plus at 13 psi) had the fewest nonunions[28].

However, recent reports have demonstrated deleterious effects of pulsed lavage on musculoskeletal tissues. Bhandari et al. reported microscopic and macroscopic damage to human

cadaver bone with the Surgilav (Stryker) pulsed lavage system at 70 psi[9]. Dirschl et al. demonstrated a delay in fracture-healing in a rabbit model after irrigation with the same device at 70 psi[10]. A recent study by the same group showed inhibition of trabecular fracture-healing after irrigation with pulsed lavage at pressures of >50 psi[11]. Those two studies used devices that produced far more pressure than the pulsed lavage that we used, and direct comparison is probably not appropriate. However, Draeger and Dahners, in a study involving bovine cadaver muscle sections, evaluated the same device that was used in the present study and found greater macroscopic destruction and organic tissue in the fluid runoff compared with bulb syringe and suction irrigation[12]. Boyd and Wongworawat tested the Surgilav system on ovine muscle and found greater cell death and deeper tissue penetration compared with gravity saline solution flow at 3 psi[13].

Wheeler et al., in a porcine dorsal wound model, showed that irrigation with high-pressure pulsed lavage increased the susceptibility to clinical infection[29]. Wounds were treated before inoculation with either pulsed lavage (70 psi) or syringe (8 psi) irrigation. After four days, wounds in both of the treated groups had greater gross evidence of infection and bacterial counts than did an untreated control. Those authors concluded that while high-pressure pulsed-lavage irrigation results in superior bacterial removal, it also results in host tissue damage and should be reserved for grossly contaminated wounds in which the benefits outweigh the adverse effects. In our study, we did not include an untreated control group, and we treated the wounds six hours after inoculation in an effort to closely mimic the clinical scenario. However, our results support the conclusions of Wheeler et al.[29].

As in all studies that use an animal model, the current study does have limitations. The goat extremity model was chosen as it more closely mimics the clinical scenario than does a smaller-animal model (that is, the goat model allows for a larger wound, similar irrigation volumes, and use of the same devices as are used clinically). The use of a luminescent bacterium and photon-capturing imaging provides immediate quantification of wound bacteria, is neither tissue-consumptive nor as susceptible to sampling error as tissue biopsies, and allows repeated measures. Unfortunately, this approach limited us to the use of gram-negative bacteria such as *Pseudomonas aeruginosa*; other species of bacteria may not produce the same results. Also, because of the large amount of trauma caused by the creation of the wound, we were not able to assess the integrity of the treated soft tissues with histological analysis; therefore, we do not have histological evidence that the pulsed lavage caused more tissue damage than did bulb-syringe lavage. However, the presumption that the greater amount of bacteria within the wounds that received pulsed lavage at forty-eight hours is due to tissue damage has been well supported by the literature. Unfortunately, the exact bacterial counts necessary to cause a clinical infection are unknown. The development of a clinical infection is dependent on a complex and poorly understood interplay of bacterial counts, bacterial virulence, and host factors. We chose to evaluate bacterial counts with a bioluminescent model with an understanding that these other factors are difficult to measure.

While continued clinical trials are needed, the current study indicates that the use of the three alternative irrigation solutions results in a more pronounced rebound of bacterial growth compared with normal saline solution in our model. These solutions may be better at initial bacterial removal, but this ability to remove bacteria may come with associated deleterious effects to the host tissue and could potentially lead to complications. ■

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